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Morphohistometric investigations of placentas of diabetic patients in correlation to the metabolic adjustment of the disease

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1 Introduction

Careful attention to maternal blood glucose levels is of paramount importance for perinatal outcome in pregnant diabetic patients. Strict metabolic supervision, adjustment of maternal metabolism is needed, and thorough fetal monitoring is absolutely essential. Yet, placental insufficiency is still much more common in diabetic than in normal pregnancies.

The histometric study presented was undertaken to investigate if the placental morphologic alterations are mainly attributable to blood glucose levels during pregnancy or rather to the severity and duration of the diabetes according to White's classification. Data from earlier histometric studies of terminal villi of placentas from diabetic mothers classified according to WHITE [29] and of placentas from normal pregnancies [19, 260 were used as controls.

2 Material and methods

This study presents data on 50 pregnant diabetic patients. The metabolic status was arbitrarily divided into two groups, named "good" and "bad", for each trimester and for the whole pregnancy. The basis for this division was the HbA_{1C} values (Table I). A micro column method for the determination of hemoglobin minor fractions of following the methods M. B. JONES et al [10] and FRIEDMAN and HUMBERT [4] was modified by HEINTZE et al [8]. In terms of reproducibility, a coefficient of variation of 5.5% in the normal range ($4.5 \pm 0.25\%$, $n = 10$) and of 4.7% in the pathologic range ($9.5 \pm 0.44\%$, $n = 10$) was found. Weight and neonatal outcome were correlated with maternal blood glucose levels during pregnancy. The individual groups of mothers did not demonstrate significant differences regarding average age, parity and weight. None of the pregnancies ended before the 37th gestation week. For morphometric evaluation the placentas were fixed in 10% formalin immediately after delivery and routinely treated [19]. From three areas of each placenta tissue was taken and sections cut. In each section 50 terminal villi from the periphery of placental cotyledon [23] were measured and the following parameters evaluated:

- surface area and circumference of the villus,
- total surface area, circumference and number of villous vessels,
- length and number of epithelial plates.

Based on the parameters measured:

- degree of vascularization and
- the villous circumference coverage by epithelial plates were determined.

For this study we used the semiautomatic electronic image analysis system Kontron-Videoplan. All

Table I. Maternal metabolic adjustment of diabetics throughout pregnancy based on HbA_{1C}

	good (1)	bad (2)
HbA_{1C} early pregnancy	$\lesssim 4.8\%$	$> 4.8\%$
HbA_{1C} second trimester	$\lesssim 4.7\%$	$> 4.7\%$
HbA_{1C} third trimester	$\lesssim 4.6\%$	$> 4.6\%$
HbA_{1C} entire pregnancy	3–4	5–6

our studies are based on the same histologic parameters, instruments, measurement proceedings and statistic calculations. The data are thus comparable with these of normal placnetas. Statistics were done by analysis of variance.

3 Results

Surface areas of the terminal villi in "good" metabolic status were 2323 ± 210 μm², in the "bad" group 2411 ± 230 μm². The data from the two groups did not differ significantly; however, they demonstrated a marked difference ($p < 0.05$) compared to the values for normal placentas (1977 ± 190 μm², Figure 1). Analogically, the circumferences measured 172 ± 12 micra in the "good" group as opposed to 180 ± 14 μm² in the "bad" group (normal values: 157 ± 8 μm², $p < 0.05$). The total surface area of the villous vessels in both groups differs significantly from that in the normal placenta (706 ± 230 μm², $p < 0.05$). These measurements demonstrate a slight but not statistically significant decrease in vascular surface area in "bad metabolic conditions" (528 ± 193 μm²), compared to that in "good metabolic conditions" (587 ± 177 μm², Figure 2). Analogous differences can be observed in the measured "vessels circumference": 161 ± 33 μm in good, 148 ± 29 μm in bad metabolic conditions, compared to 180 ± 36 μm in the healthy standard group. The number of

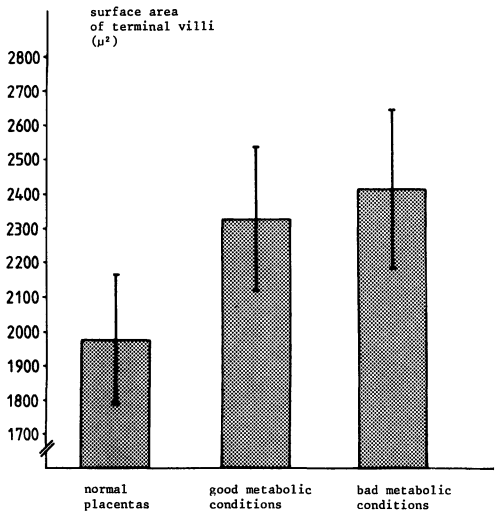

Fig. 1. Surface area of placental terminal villi in Diabetes mellitus in correlation to the maternal metabolic adjustment.

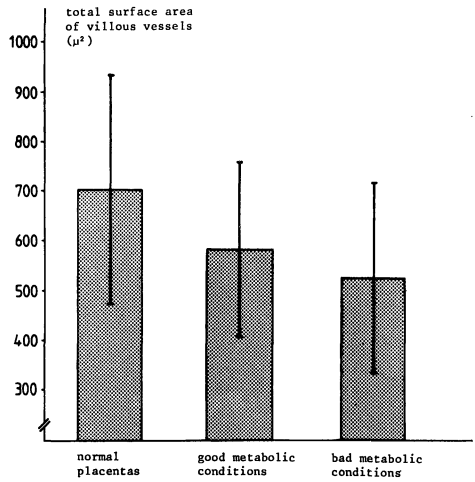

Figure 2. Total surface area of placental villous vessels in Diabetes mellitus in correlation to the maternal metabolic adjustment.

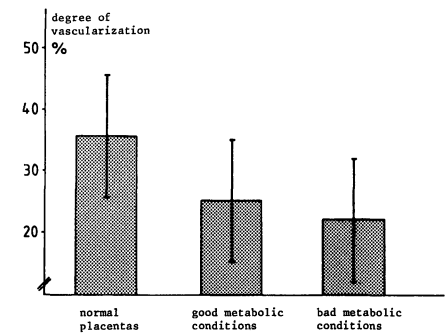

Figure 3. Degree of vascularization of placental terminal villi in Diabetes mellitus in correlation to the maternal metabolic adjustment.

vessels in the terminal villi is statistically significantly ($p < 0.01$) reduced both in good metabolic conditions (3.3 ± 0.5), compared to the normal standard of 4 ± 0.5. There is no statistical difference between the two diabetic groups. The degree of vascularization is significantly decreased ($p < 0.05$) in the diabetic group compared to the normal standard: in good metabolic conditions 25.3 ± 9% versus 35.8 ± 10% in the normal controls. In "bad" conditions it is as low as 22.1 ± 10% (Figure 3). The number of epithelial plates is significantly diminished in the diabetic groups ($p < 0.001$) irrespective of the metabolic conditions. The length and the villous circumference

Table II. Parameters of epithelial plates in placental villi in Diabetes mellitus

Parameter	Normal controls	Diabetes mellitus		
		Good metabolic adjustment	Bad metabolic adjustment	
Total length of epithelial plates	29 ± 5.4 μ	34 ± 4.1 μ	33 ± 5 μ	NS
Number of epithelial plates	2.5 ± 0.4	2.0 ± 0.3	1.8 ± 0.4	N/D = $p < 0.001$
Villous circumference coverage by epithelial plates	18.5 ± 5%	19.8 ± 4.5%	18.5 ± 6%	NS

coverage of the epithelial plates show no significant difference compared with the standard material (Table II).

Pre-eclampsia was observed in 5 pregnancies (10%), all of which had class D diabetes (White). There was no correlation with the metabolic conditions.

Seven of eleven macrosomic newborns (beyond the 90th percentile) were born to mothers with bad metabolic conditions. There was no relation to the White classification. Four of five small-for-date infants were born to mothers with a class D diabetes. There was no correlation to the metabolic conditions (Figure 4).

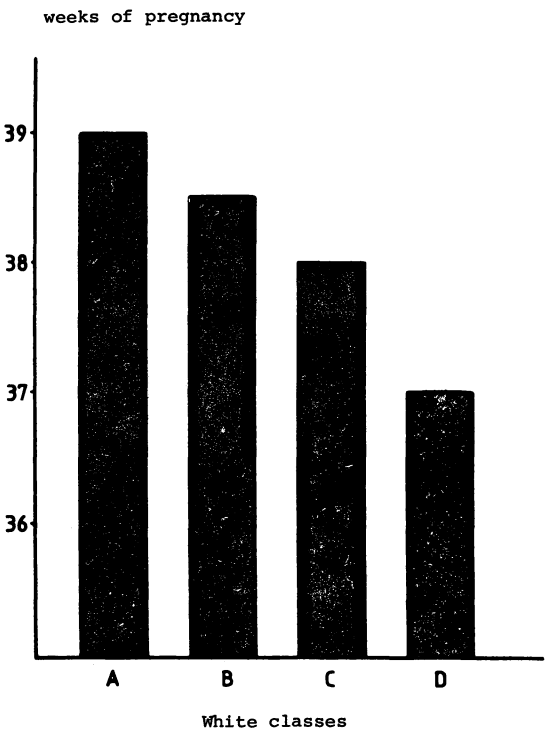

Figure 4. Termination of pregnancy in correlation with the White classes.

Four newborns showed various malformations, three of them were born to mothers with bad metabolic conditions. Apgar values and pH of the umbilical artery were comparable to the values of the normal control group. The need for premature terminations of pregnancy rose noticeably in the higher White classes. The limited number of patients precludes statistical analysis. Accordingly, we restricted ourselves to describing the clinical observations.

4 Discussion

In the majority of studies on morphology and histometry of the placenta, a retardation in the maturation of placental villi in maternal diabetes is demonstrated [3, 7, 9, 11]. There is some controversy about the question of whether these morphologic changes are related to severity and duration of the diabetes or rather to the maternal blood glucose level. In an earlier study [26] we demonstrated a statistically significant increase in size of the terminal villi in all diabetics, with a noticeable increase from group A to group C; although in group D the changes were less marked and closer to the normal standard values. These findings were in accordance with the results obtained by other investigators [24, 27, 28]. This difference between the normal and the diabetics collectively also manifests itself in the study presented here. There are no statistical differences between the placentas in "good" and "bad" metabolic conditions. Compared to earlier investigations on diabetic placentas, we now find smaller

villi. This can be explained by the fact that in the material presented there is a greater number of class A and D diabetic cases (White).

Even when comparing the data from the three patients with the worst metabolic conditions (surface area of villi 2398 μm^2, total surface area of villous vessels 535 μm^2) with those of the three diabetics with the best metabolic conditions (surface area of villi 2366 μm^2, total surface area of villous vessels 567 μm^2) no statistical difference results. Thus, our results are in marked contrast to those of BJOERG and PERSON [2] and GEPPERT et al [6]. In our material the surface area of all vessels in a villus was signifantly smaller than in the normal standard group. The differences in the degree of vascularization are more marked in the different White classes than in the two groups of metabolic conditions. TEASDALE [27] described a larger surface area of the vessels. We also noted this, but only in the White class C group. He also detected a slightly reduced degree of vascularization in the diabetic placenta. The diminuation in the number of vessels per cross section is highly significant; however, it is independent of the metabolic conditions. Like SHADMI [25] and LIN [13], we found a diminished number of the epithelial plates, but no marked difference in their length nor in the villous circumference coverage by epithelial plates.

HbA_{1C} is an important measurement for metabolic control and for retrospective evaluation. In contrast to LESLIE et al [12], but in agreement with O'SHAUGNESSY [22] and WORTH [30], we hold the view that HbA_{1C} decreases in the course of normal pregnancy. FUHRMANN [5], LOWY [14], LESLIE [12], and NELSON [18] effectively demonstrated the correlation between blood glucose levels and fetal malformations, which is being confirmed in our comparatively small study group. In the same way macrosomia was mostly observed in mothers with bad metabolic conditions (seven out of eleven newborns) in our study group; whereas, five out of seven small-for-date babies in our collective were born to mothers in the White class D group.

Many investigators agree [1, 14, 15] that the rate of complications is still higher in diabetic mothers, despite all therapeutic efforts. OLOFSSON [20, 21], in his long-time study, repeatedly observed fetal distress situations unrelated to maternal metabolic conditions. There is also an increased risk of intrauterine fetal death [1, 16, 17]. Fortunately, in our group of patients, we had no severe fetal distress or fetal death. This may be partly attributed to good monitoring, or if indicated, premature termination of the pregnancy.

Our clinical observations and histometric results lead us to draw the following conclusion: For a good fetal outcome, early and continuous metabolic control of the diabetic mother is of vital importance. By the same token, severity and duration of the disease must not be omitted from the list of risk factors when managing a diabetic pregnancy.

Abstract

In order to determination if an interdependence exists between the well-known maturational disturbances in placentas of diabetics and the quality of metabolic adjustment, a morphometric study covering 7,500 terminal villi of 50 placentas was performed. The diabetic mothers were divided into two groups according to satisfactory or unsatisfactory blood glucose values. Significant differences in placental retardation were observed between both diabetic groups and a normal control group. Although outcomes did not differ greatly between the two diabetic groups, macrosomia and the ratio of malformations in the newborns correlate with unsatisfactory metabolic management. Small-for-date babies, the need for early termination of pregnancy as well as preeclampsia are related to the severity and duration of the disease.

Keywords: Diabetes mellitus, metabolic adjustment, morphometry, placenta.

Zusammenfassung

Histometrische Untersuchungen an Plazenten von diabetischen Müttern in Korrelation zur Stoffwechseleinstellung
In einer morphometrischen Studie wurden 7500 Terminalzotten von 50 Plazenten untersucht, um herauszufinden, ob die bei mütterlichem Diabetes mellitus bekannten Plazentareifungsstörungen eine Abhängigkeit von der Stoffwechseleinstellung aufweisen. Die Diabetikerinnen wurden je nach der Qualität der Blutzuckerwerte in zwei Gruppen eingeteilt, und zwar sowohl für jedes Trimenon als auch für die gesamte Schwangerschaft. Die Grundlage für diese Einteilung bildete das HbA_{1C}, das mit Hilfe eines Mikrosäulenverfahrens ermittelt wurde.

Zuvor waren in einer Pilotstudie Normalwerte für die Schwangerschaft ermittelt worden. Für die Morphometrie nutzten wir das halbautomatische elektronische Bildanalyseverfahren Videoplan, Kontron.
Die Zottenquerschnittsflächen und ihre Umfänge unterschieden sich zwar nicht signifikant in den beiden Diabetesgruppen untereinander, wohl aber zeigten beide Kollektive signifikante Unterschiede in der Zottengröße im Vergleich zur normalen Kontrollgruppe. Während die Gesamtquerschnittsflächen und die Umfänge der Zottengefäße der Plazenten von schlecht eingestellten Müttern nur geringfügig kleiner gegenüber denen von gut eingestellten Patientinnen waren, zeigten sich ebenfalls signifikante Unterschiede zwischen den beiden Kollektiven und den normalen reifen Plazenten. Die Anzahl der Gefäße, der Vaskularisationsgrad sowie die Entwicklung der Epithelplatten waren in der selben Weise unabhängig von der Stoffwechsellage signifikant verringert. Die Frequenz kindlicher Makrosomie jedoch wie auch die Rate von Mißbildungen bei den Neugeborenen waren bei schlecht geführtem Diabetes erhöht, während das Auftreten von kindlicher Mangelentwicklung, mütterlicher Gestose und die Notwendigkeit zu vorzeitiger Entbindung mit Schwere und Dauer des Diabetes korrelierten.
Unsere klinischen Beobachtungen weisen auf die Wichtigkeit der straffen Stoffwechselführung für ein optimales fetal outcome hin, auf der anderen Seite beweisen unsere morphometrischen Ergebnisse, daß die morphologische Korrelate einer Plazentainsuffizienz bei Diabetes mellitus unabhängig von der Güte der Blutzuckerwerte auftreten.

Schlüsselwörter: Diabetes mellitus, Histometrie, Morphometrie, Plazenta, Stoffwechseleinstellung.

Résumé

Etude morphohistométrique des placentas de diabétiques en corrélation avec l'equilibre métabolique de la maladie
On a examiné de façon morphométrique les coupes de 7500 villosités terminales provenant de 50 placentas afin de déterminer s'il existe une relation entre le degré d'équilibre métabolique chez les diabétiques et les altérations morphologiques bien connues du placenta. On a divisé en deux groupes les mères diabétiques selon leur équilibre métabolique, intitulé "bon" et "mauvais" pour chaque trimestre et pour l'ensemble de la grossesse. Les valeurs de l'HbA1c ont servi de base pour cette division et ont été dosées par une méthode avec microcolonne. Pour l'étude morphométrique on a utilisé un système d'analyse d'images électroniques semi-automatique (Kontron-Vidéoplan).
Il n'y a pas de différence significative entre les deux groupes de diabétiques pour les surfaces et les circonférences des vilosités terminales. Toutefois, les deux groupes de diabétiques présentent une différence significative avec les placentas normaux, utilisés comme contrôles. L'aire totale des vaisseaux villositaires ainsi que leurs circonférences sont également significativement différentes de celles des placentas normaux. L'aire de surface vasculaire est légèrement diminuée lorsque l'équlibre métabolique est mauvais par rapport à celle des patientes ayant un bon équilibre métabolique. Le nombre de vaisseaux, le degré de vascularisation et la taille des couches épithéliales sont également significativement diminués chez les patientes diabétiques quels que soient leurs équilibres métaboliques. D'autre part, on observe plus fréquemment lors de mauvais équilibres métaboliques une macrosomie et une augmentation de la fréquence des malformation chez le nouveau-né. Il y a plus souvent survenue de retards de croissances, nécessité d'une terminaison prématurée de la grossesse et, également, apparition de "gestoses" maternelles au cours des diabètes sévères et de longues durées.
Cette étude souligne l'importance citale d'un bon contrôle du métabolisme glucidique de la mère diabétique pour le développement fœtal et son évolution. Par ailleurs, il n'y a pas de relation entre les paramètres morphologiques et l'insuffisance placentaire et ceux du métabolisme glucidique maternel.

Mots-clés: Diabète sucré, équilibre métabolique, morphométrie, placenta.

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